The association between serum levels of potential biomarkers with the presence of factors related to the clinical activity and poor prognosis in spondyloarthritis

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ABSTRACT

Background: Serum biomarkers traditionally associated with inflammatory activity and a poor prognosis in rheumatic diseases do not show the same relationship in spondyloarthritis. **Objective:** To establish the association between serum levels of potential biomarkers with the presence of factors related to clinical activity and poor prognosis in spondyloarthritis. **Methods:** Sixty-two patients were included: 13 with reactive arthritis, 19 with ankylosing spondylitis, and 30 with undifferentiated spondyloarthritis. The results were compared with those from 46 healthy controls. Clinical, radiological, and laboratory characteristics were assessed. The results were analyzed based on the presence of uveitis, enthesitis, inflammatory back pain, arthritis, HLA-B27 and sacroiliac involvement. The analyzed biomarkers included ESR, US-CRP, SAA, LBP, FSC-M, and MMP-3; and cytokine serum levels measured were: IL-17, IL-6, IL-1α, TNF-α, IFN-γ, and IL-23. **Results:** Forty-three (69.4%) patients were male. The average age was 31.9 ± 9.9 years and the age at the onset of symptoms was 26.9 ± 7.3 years. HLA-B27 was positive in 26 (41.9%) patients, inflammatory back pain in 42 (67.7%), arthritis in 44 (71.0%), and enthesitis in 34 (54.8%). IL-17, IL-23, TNF-α, IL-6, IL-1α, and US-CRP levels were significantly higher in patients with SpA when compared to controls. US-CRP (P = 0.04), IL-6 (P = 0.003), IL-1α (P = 0.03), and LBP (P = 0.03) levels were associated with presence of HLA-B27, inflammatory back pain, and arthritis. **Conclusion:** An increase in serum levels of US-CRP, IL-6, IL-1α, and LBP was correlated with factors associated with clinical activity and poor prognosis in spondyloarthritis.

Keywords: spondyloarthritis, spondyloarthropathies, reactive arthritis, ankylosing spondylitis, ankylosing spondyloarthritis, rheumatic diseases, enthesopathy, low back pain, arthritis, biomarkers.

INTRODUCTION

Spondyloarthritis (SpA) is a heterogeneous group of chronic inflammatory diseases that share clinical and radiological manifestations. They are associated with the presence of human leukocyte antigen (HLA-B27), which leads to a tendency for familial association.[1–3] The following diseases comprise SpA: ankylosing spondylitis (AS), reactive arthritis (ReA), psoriatic arthritis (PsA), arthritis associated with intestinal inflammatory disease, and undifferentiated spondyloarthirtis (uSpA).[4]

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The clinical presentation of SpA is characterized by compromised joints of the axial and peripheral skeleton, enthesitis, and extra-articular manifestations. Together with AS, uSpA is the most common subtype, with a prevalence between 0.7% and 2.0% in the general population.[3]

Disease subtype and progression over time have traditionally been correlated with prognostic factors such as race, gender, age at onset, HLA-B27, and early axial skeleton involvement.[5–7]

In studies conducted in Latin America, the most frequent types of SpA presented were uSpA and ReA. The initial stages of these diseases are associated with joint inflammatory compromise and enthesitis of the lower limbs.[7–9]

Some characteristics related to the onset of the disease, such as age at onset, HLA-B27, symptom duration of the first episode, and male gender, among others, may determine the clinical expression and evolution of SpA. In general, men present more severe forms and have greater axial compromise, while women have a greater peripheral joint compromise and less sacroiliitis.[2] In juvenile AS, hip joint compromise is considered a factor for poor long-term prognosis.[9] Other markers of disease severity include erythrocyte sedimentation rate (ESR) > 30 mm/hr, poor response to non-steroidal anti-inflammatory drugs (NSAID), limited range of motion, limitation of the lumbar spine, dactylitis, oligoarthritis, and age at onset of less than 16 years.[10]

A genetic predisposition exists for the development of the disease, as evidenced by its strong association with HLA-B27, especially in the case of AS, where 90% of the patients are positive for this allele.[11] Patients with SpA and HLA-B27 have more severe and prolonged joint symptoms, greater axial compromise of the hips, and more frequent extra-articular manifestations, such as uveitis and cardiac involvement.

Recently, genomic studies of patients with AS have identified and validated other loci aside from the HLA-B27 that are involved in the pathogenesis of this disease. These genes include the endoplasmic reticulum-associated aminopeptidase 1 (ERAP-1), IL-23 receptor (IL-23R), IL-1 receptor (IL-1RII), and two loci that code for unknown genes. The risk attributed to populations with the three known associated genes is 90%, 26%, and 1%, respectively.[12–14]

The main challenges for the management of SpA are related to the lack of biomarkers associated with disease activity as well as the inability to predict joint damage and response to treatment. Recent studies have focused on the possible contribution of soluble biological markers that have been selected based on the current understanding of their role in inflammation and/or their association with joint matrix remodeling.[15]

Biomarkers may provide information that promotes understanding of the prognosis, activity of the disease, and pathogenesis of SpA.[16]

ESR and C-reactive protein (PCR) are two currently used biomarkers for the evaluation of inflammatory activity of the disease. However, these biomarkers do not have the most ideal specificity, sensitivity, and reproducibility characteristics. These inflammation markers offer a poor correlation to the degree of activity in patients with SpA.[17]

Recently, other biomarkers for SpA activity have been proposed, including metalloproteinase 3 (MMP-3),[18] IL-1α,[19] IL-6,[20] macrophage colony stimulating factor (M-CSF),[21] and lipopolysaccharide-binding protein (LBP).[22] IL-17 is among the recently evaluated biomarkers, but no association with the disease's activity has been reported.[12] However, significantly elevated serum levels of IL-17 and IL-23 have been recently described in patients with AS *versus* healthy controls, suggesting that these two cytokines play critical roles in the pathogenesis of AS.[23]

The objective of this study was to establish the association between potential SpA biomarkers with the presence of factors associated with activity and poor prognosis in patients at the early stages of the disease.

PATIENTS AND METHODS

Blood samples were taken from 62 patients with a diagnosis of SpA according to the classification criteria established by the European Spondyloarthritis Study Group (ESSG).[24] Of these patients, 43 were men and 19 were women, and the patients were collected as convenient and in a consecutive manner. The patients were attended the SpA Clinic of the Hospital Militar Central between January 2010 and May 2011. Thirteen patients were diagnosed with ReA based on the proposal for diagnosis described at the Third International Workshop on ReA in Berlin,[25] nineteen patients were diagnosed with AS based on the New York modified criteria, and 30 patients were diagnosed with uSpA according to ESSG classification criteria. At the time of the study, all patients received NSAID and sulfasalazine (1.5–2 g/day). None of the patients received treatment with biological, intra-articular, or systemic glucocorticoids therapy. Forty-six healthy individuals were included in the study as controls. The serum samples of the participating healthy controls were obtained from the bank of the Central Military Hospital from subjects without inflammatory, autoimmune or infectious diseases, and gender and age were taken into account.

The stage of the activity of the disease was measured by the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI),[26] and the functional state was evaluated through

the Bath Ankylosing Spondylitis Functional Index (BASFI).[27] All measures related to disease activity and physical function of patients were made as recommended by the Assessment of SpondyloArthritis International Society (ASAS).[28] All of the patients received non-steroidal anti-inflammatory drugs and sulfasalazine, but none of them received biological therapy or intra-auricular or systemic corticoids.

Serum samples

Serum samples were prepared from 3 mL venous blood without anticoagulant according to standard technique. All samples (sera from patients with SpA and healthy subjects) were centrifuged for 10 minutes to 2,500 rpm to be subsequently frozen at –80º C until their assessment with a time interval not exceeding two months after obtaining each. Serum samples from patients and controls were collected and processed into time periods simultaneously. Also, the blood samples were collected simultaneously with the clinical activity parameters.

Analysis of flow cytometry

A Cytometric Bead-Array (CBA Flex Set) was used to measure the serum cytokine levels (IL-17, IL-6, IL-1α, TNF-α, and INF-γ). The capture beads, detection antibodies conjugated with PE, controls and serum samples from patients and healthy controls were incubated together to form sandwich complexes. Samples were collected using a FACS Canto II flow cytometer™. The data were acquired with the FACS DIVA software, and the results were generated in graphical and tabular format using BD FCAP software, creating a marker gate based on 1,800 control events for each cytokine and the levels are expressed as means ± standard deviations (SD) in pg/mL.

Enzyme-linked immune-sorbent assay (ELISA)

Serum levels were determined for IL-23, MMP3, SAA and M-CSF (R & D Systems), through ELISA using paired antibodies according to manufacturer's recommendations. Serum samples were analyzed in duplicate. The values for each cytokine in a group of subjects are expressed as means ± SD in pg/mL.

Ultra-sensitive C-reactive protein (US-CRP) and LBP levels were analyzed by chemiluminescence. Comparisons between samples were conducted on the same day. The reference value which was considered positive the US-CRP was 0.9 mg/dL.

The project was conducted under the principles of the Helsinki Declaration and was approved by the institution's ethics committee. All of the participants had previously signed informed consent, and confidentiality was maintained.

Statistical analysis

The statistical package SPSS 17.0 for Windows was used to analyse the data. For the assessment of continuous variables, measures of central tendency and dispersion were used; for comparison between groups of quantitative variables with parametric distribution was used the Student's *t* test for independent samples. The categorical variables were presented in frequency charts and percentages, and the Chi-squared test and Fisher's exact test were used, if necessary, for comparing groups. A P-value < 0.05 was considered statistically significant. The distribution by gender and average age of patients and controls were taken into account, but paired analysis was not performed.

RESULTS

General characteristics of the population

Demographic data, general information, and characteristics related to the disease of all 62 patients are shown in Table 1.

History related with the onset of disease

The most frequent symptoms at the onset of the disease were arthritis and inflammatory lower back pain, followed by enthesopathy (Table 2).

Table 1
Demographic data (spondiloarthritis, n = 62)

Age* (years)	31.9 ± 9.9
Age at onset of symptoms* (months)	26.9 ± 7.3
Evolution time* (years)	5.01 ± 5.7
Gender, M (%)	43 (69.4)
Gender ratio M:F	3:1
HLA-B27 (+)	26 (41.9)
AS	20 (32)
uSpA	29 (47)
ReA	13 (21)

*Mean ± SD.
AS: ankylosing spondylitis; uSpA: undifferentiated spondyloarthirtis; ReA: reactive arthritis.

Table 2
Symptoms present at the onset of disease (SpA n = 62)

Inflammatory back pain	67.7%
Arthritis	71.0%
Enthesitis	54.8%
Infection – diarrhea	29.0%
Uveitis – anterior	12.9%
Gluteal pain	17.7%
Dactylitis	19.4%

Disease activity

At the time of patient evaluation, disease activity was classified as moderate or severe for the majority of the patients (moderate activity if BASDAI was between 4–6.9 and severe if BASDAI ≥ 7). The pattern of disease compromise was distributed as follows: 38 (61.3%) patients had peripheral involvement, seven (11.3%) patients had axial involvement, and four (6.5%) patients had mixed involvement (Table 3).

The different quantified serum markers in patients and the healthy controls are described in Tables 4 and 5. Within each serum marker associated with inflammation: IL-17, IL-23, TNF-α, IL-6, IL-1α, and US-CRP had statistically significant differences compared to the healthy controls, and cytokine levels were higher in patients with SpA. IFN-γ, MMP-3, SAA, and M-CSF levels were also higher in patients with SpA when compared to the control sample, but these differences were not statistically significant (Table 4). The data for activity of clinical and biological markers are in the Table 5.

The different inflammation markers were correlated with factors of poor prognosis at the onset of disease, including HLA-B27 and the precedents of Inflammatory Back Pain and arthritis, as shown in Table 6. The expression of markers, such as US-CRP (P = 0.04), IL-6 (P = 0.003), IL-1α (P = 0.03), and LBP (P = 0.03), was significantly higher in patients who presented with factors of poor prognosis associated with the onset of disease versus those patients who did not present poor prognosis factors (Table 6).

Table 3
Clinical variables related to disease activity

Disease activity, determined by examiner EVA 0–10*	5.4 ± 2.0
Disease activity, determined by patient EVA 0–10*	6.5 ± 2.4
Morning stiffness (min)	46.3 ± 35.7
Schober test**	4.9 ± 5.5
Thoracic expansion* (cm)	4.2 ± 1.3
Occiput to wall distance* (cm)	0.05 ± 0.4
Patrick test**	27 (43.5)
Peripheral involvement**	38 (61.3)
Axial involvement**	7 (11.3)
Mixed involvement**	4 (6.5)
BASDAI*	6.1 ± 2.0
BASFI*	5.8 ± 2.3
US-CRP (mg/L)*	9.4 ± 16.5
ESR (mm/hr)*	13.5 ± 13.8

*Mean ± SD; **Frequency n (%).

US-CRP: ultrasensitive C-reactive protein; ESR: erythrocyte sedimentation rate.

Table 4
Comparison of inflammation serum markers in spondiloarthritis patients and healthy controls

Marker	SpA (n = 62)	Controls (n = 46)	P
IL-17 (pg/mL)	52.54 ± 87.12	13.73 ± 26.40	0.000
IL-23 (pg/mL)	4.76 ± 2.93	3.12 ± 0.717	0.000
TNF-α (pg/mL)	24.20 ± 36.35	15.95 ± 12.67	0.000
IL6 (pg/mL)	48.24 ± 73.73	20.14 ± 4.56	0.000
IFN-γ (pg/mL)	0.88 ± 2.95	0.56 ± 1.22	0.615
IL-1α (pg/mL)	46.0 ± 23.22	42.23 ± 30.84	0.001
MMP-3 (ng/mL)	21.42 ± 21.83	18.05 ± 9.96	0.900
SAA (ng/mL)	853.7 ± 946.2	282.49 ± 371.94	0.001
M-CSF (pg/mL)	102.48 ± 67.86	34.74 ± 33.40	0.001
LBP (µg/mL)	7.54 ± 3.71	3.5 ± 1.8	0.045
ESR (mm/hr)	17.08 ± 13.87	3.8 ± 0.7	0.003
US-CRP (mg/L)	8.31 ± 16.7	1.13 ± 0.88	0.020

Results are expressed as means.
IL: interleukin; TNF-α: tumor necrosis factor-alpha; INF-γ: interferon gamma; MMP-3: metalloproteinase 3; SAA: serum amyloid A; M-CSF: monocyte colony stimulating factor; LBP: lipopolysaccharide-binding protein; ESR: erythrocyte sedimentation rate; US-CRP: ultra-sensitive C-reactive protein; SpA: spondyloarthritis.

Table 5
Activity data in SpA Group and subtypes

	SpA	AS	uSpA	ReA
BASDAI	6.1 ± 2.0	6.4 ± 2.0	6.2 ± 1.6	5.7 ± 2.7
BASFI	5.8 ± 2.3	5.4 ± 2.3	5.9 ± 2.1	5.9 ± 2.7
ESR (mm/hr)	17.1 ± 13.8	13.4 ± 12.8	15.2 ± 10.4	26.7 ± 18.1
US-CRP (mg/L)	8.31 ± 16.7	7.9 ± 16.4	4.5 ± 9.9	22.4 ± 22.2
LBP (µg/mL)	7.5 ± 3.7	0.53 ± 2.4	6.6 ± 3.5	10.0 ± 4.8
SAA(ng/mL)	853.7 ± 946.2	752.5 ± 871.8	652.0 ± 820.2	1459.7 ± 1124.7

Results are expressed as means ± SD.
SpA: spondyloarthritis; AS: ankylosing spondylitis; uSpA: undifferentiated spondyloarthritis; ReA: reactive arthritis; ESR: erythrocyte sedimentation rate; US-CRP: ultra-sensitive C-reactive protein; LBP: lipopolysaccharide-binding protein; SAA: serum amyloid A. .

Table 6
Factors of poor prognosis in patients with spondyloarthritis: HLA-B27+, IBP, and arthritis. Description of the construct of poor prognosis as group variables

	Factors (+) n = 9	Factors (-) n = 53	P
ESR (mm/hr)	21.6 ± 16.3	16.3 ± 13.6	0.4
US-CRP (mg/L)	21.1 ± 26.0	7.4 ± 13.9	0.04
SAA (ng/mL)	1312.9 ± 1184.8	786.6 ± 894.9	0.4
MMP-3 (ng/mL)	29.4 ± 32.6	20.1 ± 19.8	0.7
M-CSF (pg/mL)	87.2 ± 33.1	106.5 ± 71.9	0.9
IL-6 (pg/mL)	79.8 ± 55.3	43.3 ± 76.2	0.003
IL-1α (pg/mL)	56.1 ± 30.6	43.7 ± 21.7	0.03
TNF-α (pg/mL)	19.4 ± 5.4	24.7 ± 39.6	0.5
IL-17 (pg/mL)	69.3 ± 69.1	49.3 ± 90.9	0.06
IL-23 (pg/mL)	4.9 ± 2.9	4.8 ± 3.0	0.8
INF-γ (pg/mL)	0.6 ± 1.2	0.9 ± 3.2	0.68
LBP (µg/mL)	9.9 ± 4.5	7.2 ± 3.4	0.03

Results are expressed as means ± SD. Statistically significant p-values in patients with SpA against factors of poor prognosis ($P < 0.05$).
IBP: inflammatory back pain; ESR: erythrocyte sedimentation rate; US-CRP: ultra-sensitive C-reactive protein; SAA: serum amyloid A; MMP-3: metalloproteinase 3; M-CSF: monocyte colony stimulating factor; IL: interleukin; TNF-α: tumor necrosis factor-alpha; INF-γ: interferon gamma; LBP: lipopolysaccharide-binding protein.

DISCUSSION

The current study compared blood cytokine levels in a population of patients with SpA at its early stages (less than five years of evolution) with a group of healthy controls. IL-17, IL-23, TNF-α, IL-6, IL-1α, and US-CRP levels were higher in patients with SpA than in healthy controls. Also, US-CRP, IL-6, IL-1α, and LBP levels were significantly elevated in patients with factors associated with poor prognosis, such as HLA-B27, inflammatory lower back pain, and arthritis, when compared with patients without factors for poor prognosis.

The majority of studies related to inflammatory markers in SpA have been conducted in populations of patients with AS; in contrast, the population of this study included patients with ReA and uSpA in addition to AS.[29,30]

In our study, US-CRP levels were higher in patients with SpA as compared to healthy controls, showing a correlation with the presence of factors of poor prognosis. In AS, only 40%–60% of the patients have elevated. Patients without elevated values may have clinically active disease, which generally points to a poor correlation between the levels of this protein and the clinical activity of the disease in AS. The standard CRP levels were compared with US-CRP levels as parameters of activity measurement in a group of patients with AS who belonged to the German cohort of SpA. US-CRP was better correlated than standard CRP with clinical parameters of disease activity in patients with axial SpA.[31] As a result, US-CRP could be better than standard CRP for evaluating disease activity in axial SpA.

IL-6 is one of the main cytokines that have been proposed as biomarkers in SpA and is significantly elevated in the population of the current study when compared with the healthy controls. It is a pleiotropic cytokine that is well known for inducing synthesis of various hepatic proteins. Elevated IL-6 levels are found in patients with AS when compared to healthy individuals, thus revealing a correlation between IL-6 and vertebral ankylosis and disease activity.[32] Increased levels of this cytokine have also been found in patients with SpA, and a decrease of the levels are seen at two weeks post-treatment in patients who are responsive to treatment, as well as persistent reductions at a three- year follow-up. For these reasons, IL-6 is considered to be a cytokine with potential value for monitoring disease activity and response to treatment in patients with SpA.[33] The main differences with the population in the current study are that the previous study was only conducted in patients with AS and PsA, while this study included patients with AS, ReA, and uSpA. Likewise, patients in the previous study received biological therapy, but in our study, patients were not exposed to such treatment. IL-6 also showed a correlation with BASDAI scoring and Magnetic Resonance Image in terms of inflammation in a phase III trial of infliximab. Significant decreases were observed in the levels of IL-6 and of other markers following the use of infliximab when compared to a placebo. In addition, IL-6 levels were correlated to the number of inflamed peripheral joints.[34]

Recently, the role of IL-23 and IL-7 in the pathogenesis of SpA have garnered considerable interest because genetic studies have showed associations with polymorphisms in the receptor of IL-23 in AS and Crohn's disease. IL-23 induces the polarization of virgin CD4 T-cells in T helper 17 cells (Th-17), which leads to the production of IL-17, a pro-inflammatory cytokine found at high levels in the serum and synovial fluid of patients with SpA and rheumatoid arthritis (RA).[15] Recently, two studies supported the role of IL-17 in the pathogenesis of SpA in human beings.

In one of the studies, IL-17 levels were elevated in patients with established and active AS, as compared to the healthy controls, correlating with disease activity. Similar to our study population, the patients did not receive immune-modulator drugs or drugs for the modification of bone metabolism. The mean age (42 *vs*. 31 years, respectively) and the average duration of the disease (14 *vs*. 5 years, respectively) were greater

than that of our study. IL-17 serum levels were greater in patients *versus* controls.[35]

In a second study, IL-17 synovial levels were greater in patients with ReA and uSpA in comparison to patients with RA and osteoarthritis.[36] However, in a study of patients with SpA, the serum levels were greater than in the controls, and there was no correlation with disease activity or reduction with anti-TNF-α therapy.[37] A recent study of patients with AS showed that serum levels of IL-17 and IL-23 were significantly higher in patients with AS compared to healthy controls. However, no association was found between serum levels of IL-17 and IL-23 with clinical activity and laboratory parameters.[23]

Another cytokine that was elevated in the study population compared to the control population was IL-1α. Limited information exists regarding the role of this cytokine as a marker of inflammation in SpA. The evaluation of this and other cytokines, such as TGF-β, IFN-γ, and IL-10, has not been very informative.[15] A study that identified which acute phase reactants and cytokines would be useful for monitoring therapy with infliximab in AS analyzed 22 cytokines and showed that serum IL-1α distinguished patients responsive to treatment at week 6 with a sensitivity of 84.9% and specificity of 53.8%. Serum IL-1α was probably generated from the joint compartments because the synovial fluid levels were greater than the corresponding serum levels. Therefore, this cytokine is regarded as a potential biomarker in SpA.[19]

LBP is an endotoxin-binding protein that functions in a coordinated manner to facilitate the total host response against Gram-negative bacterial infections. Its structure, function, and mobilization allow for its highly sensitive pro-inflammatory response against small amounts of bacteria at the onset of the bacterial infection, and later, this response allows for the efficient elimination of viable bacteria and their remains, as well as the elimination of inflammation derived from the endotoxin.[38] This protein is included in the group of proposed diagnostic or activity biomarkers of SpA.[32] In this study, we found significantly elevated levels of LBP in patients who presented with poor prognosis factors at the onset of the disease.

Recently, an additional acute phase reactant, SAA, has been shown to be elevated in SpA and correlated with CRP, ESR, and BASDAI.[15] In our study population, SAA levels were higher in patients with SpA than in the controls. This protein is a member of the apolipoprotein family, and it is primarily synthesized in the liver and synovial fluid by monocytes and activated macrophages.[32,39] Its relationship with SpA disease activity was evaluated in a study of patients with AS and in conjunction with ESR and CRP, which were compared with BASDAI. There was a good correlation between SAA and ESR, CRP, and BASDAI, and therefore, the authors proposed it as a candidate for activity biomarker.[40] Thus, SAA is among the acute phase reactants that best predict treatment response in SpA, along with CRP and IL-6.[15] A combination of elevated basal levels of CRP and SAA show a greater predictive value for clinical response (81%) in patients with AS treated with anti-TNF.[41]

Our study documented elevated serum concentration levels of MMP-3 in patients with SpA compared to healthy individuals. An important data set has evaluated serum MMP-3 as a biomarker that reflects disease activity because it is expressed in a variety of cells within the joint, such as macrophages, fibroblasts, and chondrocytes, and in response to various stimuli and pro-inflammatory cytokines, such as TNF-α.[42]

In a recent study, a weak correlation was observed with CRP, but not with BASDAI, at the beginning of the study.[43] Weak correlations have also been found between the changes in MMP-3 and the changes in CRP and BASDAI in patients who received adalimumab.[44] In other reports, no correlation was found between MMP-3 and ESR or BASDAI.[45,46] These discrepancies may reflect the phenotype of the disease, specially its prevalence relative to active peripheral inflammation in the different cohorts. In particular, a significant correlation has been shown between MMP-3 serum levels and the histopathological degree of knee synovial inflammation in patients with predominantly peripheral SpA.[47] On the other hand, the decrease of MMP-3 in synovial fluid and serum is proportional to the decrease in the degree of inflammation in the histopathology, following treatment with infliximab. Likewise, MMP-3 is a significant independent predictor of radiographic progression in patients with AS, particularly in those patients with pre-existing radiographic damage.[43]

Important challenges still exist in the field of SpA. First, the evaluation of disease severity, especially the degree of inflammation, is hindered by the lack of sensitivity and specificity of the signs and symptoms. Likewise, biomarkers routinely used in clinical practice, such as CRP and ESR, lack sensitivity and specificity for SpA; in addition, the evaluation of inflammation through magnetic resonance image (MRI) is expensive, and access to experts with experience in its interpretation is not widely available.

Second, prognosis evaluation is hindered by the slow progression of radiographic changes, because at least two years of follow-up are required before a change can be reliably detected. However, some patients do progress rapidly. The capacity for predicting progression is limited, and the current data only support baseline scoring of radiographic

damage and inflammation detected by MRI as predictors of future progression.

Third, the evaluation of treatment response predictors has identified age, baseline functional status, CRP, and MRI score for inflammation; however, the predictive capacity of these parameters is limited. Finding better predictive parameters is an important need that has not been satisfied, because biological therapy is costly, and approximately 40% of patients do not respond to it.[48,49]

During the last few years, the use of soluble biomarkers detectable in peripheral blood and urine to address the challenges in this field has increased. However, no reports have found a group of biomarkers that can closely predict poor prognosis in patients with early stages of the disease, which is the main contribution of this study in the field of SpA.

CONCLUSIONS

The increase in blood levels of US-CRP, IL-6, IL-1α, and LBP are correlated with the presence of poor prognosis factors and persistent inflammation found in the early stages of SpA.

REFERENCES

1. Khan MA. Espondilite anquilosante: introductory comments on its diagnosis and treatment. Ann Rheum Dis 2002; 61 Suppl 3:iii3–7.
2. Sieper J, Braun J, Rudwaleit M, Boonen A, Zink A. Ankylosing spondylitis: an overview. Ann Rheum Dis 2002; 61 Suppl 3:iii8–18.
3. Zochling J, van der Heijde D, Burgos-Vargas R, Collantes E, Davis JC Jr., Dijkmans B *et al*. ASAS/EULAR recommendations for the management of ankylosing spondylitis. Ann Rheum Dis 2006; 65(4):442–52.
4. Zeidler H, Mau W, Khan MA. Undifferentiated spondyloarthropathies. Rheum Dis Clin North Am 1992; 18(1):187–202.
5. Khan MA, Kushner I, Braun WE. Comparison of clinical features in HLA-B27 positive and negative patients with ankylosing spondylitis. Arthritis Rheum 1977; 20(4):909–12.
6. Burgos-Vargas R, Vázquez-Mellado J, Cassis N, Duarte C, Casarín J, Cifuentes M *et al*. Genuine ankylosing spondylitis in children: a case-control study of patients with early definite disease according to adult onset criteria. J Rheumatol 1996; 23(12):2140–7.
7. Londoño J, González L, Ramírez A, Santos P, Avila L, Santos A *et al*. Caracterización de las espondiloartropatías y determinación de factores de mal pronostico en una población de pacientes colombianos. Rev Colomb Reumatol 2005; 12(3):195–207.
8. Burgos-Vargas R, Vázquez-Mellado J. The early clinical recognition of juvenile-onset ankylosing spondylitis and its differentiation from juvenile rheumatoid arthritis. Arthritis Rheum 1995; 38(6):835–44.
9. Burgos-Vargas R, Pacheco-Tena C, Vázquez-Mellado J. Juvenile-onset spondyloarthropathies. Rheum Dis Clin North Am 1997; 23(3):569–98.
10. Amor B, Santos RS, Nahal R, Listrat V, Dougados M. Predictive factors for the longterm outcome of spondyloarthropathies. J Rheumatol 1994; 21(10):1883–7.
11. Khan MA, Kellner H. Immunogenetics of spondyloarthropathies. Rheum Dis Clin North Am 1992; 18(4):837–64.

12. Layh-Schmitt G, Colbert RA. The interleukin-23/interleukin-17 axis in spondyloarthritis. Curr Opin Rheumatol 2008; 20(4):392–7.
13. Brown MA. Genetics of ankylosing spondylitis. Curr Opin Rheumatol 2010; 22(2):126–32.
14. Reveille JD, Sims AM, Danoy P, Evans DM, Leo P, Pointon JJ *et al*. Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. Nat Genet 2010; 42(2):123–7.
15. Maksymowych WP. Biomarkers in spondyloarthritis. Curr Rheumatol Rep 2010; 12(5):318–24.
16. Maksymowych WP. What do biomarkers tell us about the pathogenesis of ankylosing spondylitis? Arthritis Res Ther 2009; 11(1):101.
17. Spoorenberg A, van der Heijde D, de Klerk E, Dougados M, de Vlam K, Mielants H *et al*. Relative value of erythrocyte sedimentation rate and C-reactive protein in assessment of disease activity in ankylosing spondylitis. J Rheumatol 1999; 26(4):980–4.
18. Chen CH, Lin KC, Yu DT, Yang C, Huang F, Chen HA *et al*. Serum matrix metalloproteinases and tissue inhibitors of metalloproteinases in ankylosing spondylitis: MMP-3 is a reproducibly sensitive and specific biomarker of disease activity. Rheumatology (Oxford) 2006; 45(4):414–20.
19. Romero-Sánchez C, Robinson WH, Tomooka BH, Londoño J, Valle-Oñate R, Huang F *et al*. Identification of acute phase reactants and cytokines useful for monitoring infliximab therapy in ankylosing spondylitis. Clin Rheumatol 2008; 27(11):1429–35.
20. Bal A, Unlu E, Bahar G, Aydog E, Eksioglu E, Yorgancioglu R. Comparison of serum IL-1 beta, sIL-2R, IL-6, and TNF-alpha levels with disease activity parameters in ankylosing spondylitis. Clin Rheumatol 2007; 26(2):211–5.
21. Yang C, Gu J, Rihl M, Baeten D, Huang F, Zhao M *et al*. Serum levels of matrix metalloproteinase 3 and macrophage colony-stimulating factor 1 correlate with disease activity in ankylosing spondylitis. Arthritis Rheum 2004; 51(5):691–9.
22. Heumann D, Bas S, Gallay P, Le Roy D, Barras C, Mensi N *et al*. Lipopolysaccharide binding protein as a marker of inflammation in synovial fluid of patients with arthritis: correlation with interleukin 6 and C-reactive protein. J Rheumatol 1995; 22(7):1224–9.
23. Mei Y, Pan F, Gao J, Ge R, Duan Z, Zeng Z *et al*. Increased serum IL-17 and IL-23 in the patient with ankylosing spondylitis. Clin Rheumatol 2011; 30(2):269–73.
24. Dougados M, van der Linden S, Juhlin R, Huitfeldt B, Amor B, Calin A *et al*. The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. Arthritis Rheum 1991; 34(10):1218–27.
25. Kingsley G, Sieper J. Third International Workshop on Reactive Arthritis. 23-26 September 1995, Berlin, Germany. Report and abstracts. Ann Rheum Dis 1996; 55(8):564–84.
26. Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. J Rheumatol 1994; 21(12):2286–91.
27. Calin A, Jones SD, Garrett SL, Kennedy LG. Bath Ankylosing Spondylitis Functional Index. Br J Rheumatol 1995; 34(8):793–4.
28. van der Heijde D, Calin A, Dougados M, Khan MA, van der Linden S, Bellamy N. Selection of instruments in the core set for DC-ART, SMARD, physical therapy, and clinical record keeping in ankylosing spondylitis. Progress report of the ASAS Working Group. Assessments in Ankylosing Spondylitis. J Rheumatol 1999; 26(4):951–4.
29. de Vlam K. Soluble and tissue biomarkers in ankylosing spondylitis. Best Pract Res Clin Rheumatol 2010; 24(5):671–82.
30. Chen HA, Chen CH, Liao HT, Lin YJ, Chen PC, Chen WS *et al*. Factors associated with radiographic spinal involvement and hip involvement in ankylosing spondylitis. Semin Arthritis Rheum 2011; 40(6):552–8.
31. Poddubnyy DA, Rudwaleit M, Listing J, Braun J, Sieper J. Comparison of a high sensitivity and standard C reactive protein measurement in patients with ankylosing spondylitis and non-radiographic axial spondyloarthritis. Ann Rheum Dis 2010; 69(7):1338–41.
32. Romero-Sánchez C, Londoño J, De Ávila J, Valle-Oñate R. Biomarkers for spondyloarthropathies. State of the art. Rev Med Chil 2010; 138(9):1179–85.
33. Pedersen SJ, Hetland ML, Sørensen IJ, Ostergaard M, Nielsen HJ, Johansen JS. Circulating levels of interleukin-6, vascular endothelial growth factor, YKL-40, matrix metalloproteinase-3, and total aggrecan in spondyloarthritis patients during 3 years of treatment with TNFα inhibitors. Clin Rheumatol 2010; 29(11):1301–9.
34. Visvanathan S, Wagner C, Marini JC, Baker D, Gathany T, Han J *et al*. Inflammatory biomarkers, disease activity and spinal disease measures in patients with ankylosing spondylitis after treatment with infliximab. Ann Rheum Dis 2008; 67(4):511–7.
35. Wendling D, Cedoz JP, Racadot E, Dumoulin G. Serum IL-17, BMP-7, and bone turnover markers in patients with ankylosing spondylitis. Joint Bone Spine 2007; 74(3):304–5.
36. Singh R, Aggarwal A, Misra R. Th1/Th17 cytokine profiles in patients with reactive arthritis/undifferentiated spondyloarthropathy. J Rheumatol 2007; 34(11):2285-90.
37. Wendling D, Cedoz JP, Racadot E. Serum and synovial fluid levels of p40 IL12/23 in spondyloarthropathy patients. Clin Rheumatol 2009; 28(2):187–90.
38. Weiss J. Bactericidal/permeability-increasing protein (BPI) and lipopolysaccharide-binding protein (LBP): structure, function and regulation in host defence against Gram-negative bacteria. Biochem Soc Trans 2003; 31(Pt 4):785–90.
39. Uhlar CM, Whitehead AS. Serum amyloid A, the major vertebrate acute-phase reactant. Eur J Biochem 1999; 265(2):501–23.
40. Lange U, Boss B, Teichmann J, Klör HU, Neeck G. Serum amyloid A – an indicator of inflammation in ankylosing spondylitis. Rheumatol Int 2000; 19(4):119–22.
41. de Vries MK, van Eijk IC, van der Horst-Bruinsma IE, Peters MJ, Nurmohamed MT, Dijkmans BA *et al*. Erythrocyte sedimentation rate, C-reactive protein level, and serum amyloid a protein for patient selection and monitoring of anti-tumor necrosis factor treatment in ankylosing spondylitis. Arthritis Rheum 2009; 61(11):1484–90.
42. Zhu J, Yu DT. Matrix metalloproteinase expression in the spondyloarthropathies. Curr Opin Rheumatol 2006; 18(4):364–8.
43. Maksymowych WP, Landewé R, Conner-Spady B, Dougados M, Mielants H, van der Tempel H *et al*. Serum matrix metalloproteinase 3 is an independent predictor of structural damage progression in patients with ankylosing spondylitis. Arthritis Rheum 2007; 56(6):1846–53.
44. Maksymowych WP, Rahman P, Shojania K, Olszynski WP, Thomson GT, Ballal S *et al*. Beneficial effects of adalimumab on biomarkers reflecting structural damage in patients with ankylosing spondylitis. J Rheumatol 2008; 35(10):2030–7.

45. Woo JH, Lee HJ, Sung IH, Kim TH. Changes of clinical response and bone biochemical markers in patients with ankylosing spondylitis taking etanercept. J Rheumatol 2007; 34(8):1753–9.
46. Appel H, Janssen L, Listing J, Heydrich R, Rudwaleit M, Sieper J. Serum levels of biomarkers of bone and cartilage destruction and new bone formation in different cohorts of patients with axial spondyloarthritis with and without tumor necrosis factor-alpha blocker treatment. Arthritis Res Ther 2008; 10(5):R125.
47. Vandooren B, Kruithof E, Yu DT, Rihl M, Gu J, De Rycke L *et al*. Involvement of matrix metalloproteinases and their inhibitors in peripheral synovitis and down-regulation by tumor necrosis factor alpha blockade in spondylarthropathy. Arthritis Rheum 2004; 50(9):2942–53.
48. Vastesaeger N, van der Heijde D, Inman RD, Wang Y, Deodhar A, Hsu B *et al*. Predicting the outcome of ankylosing spondylitis therapy. Ann Rheum Dis 2011; 70(6):973-81.
49. Pedersen SJ, Sørensen IJ, Lambert RG, Hermann KG, Garnero P, Johansen JS *et al*. Radiographic progression is associated with resolution of systemic inflammation in patients with axial spondylarthritis treated with tumor necrosis factor αinhibitors: a study of radiographic progression, inflammation on magnetic resonance imaging, and circulating biomarkers of inflammation, angiogenesis, and cartilage and bone turnover. Arthritis Rheum 2011; 63(12):3789–800.